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[COMMUNICATION]

Innervation Pattern of Some Tonic Muscles in the Uropod of the Crayfish, *Procambarus clarkii*

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ABSTRACT—In the crayfish uropod, innervation of tonic muscles whose motor neurones travel in either the second or the third root of the sixth abdominal ganglion was investigated electrophysiologically. One or more excitatory motor neurone(s) of some muscles were in the second root and those of others were in the third root. A few muscles were innervated by two excitatory motor neurones, one of which was in the second root and another was in the third root. The second root contained no inhibitory motor neurone innervating the tonic muscles. All of the inhibitory motor neurones were in the third root.

INTRODUCTION

The crayfish muscles are prinicipally divided into two types, fast muscles and slow muscles, on the bases of physiological, biochemical and morphological properties [1, 2]. Electrophysiologically phasic and tonic muscles are correspond to fast and slow muscles respectively. The phasic muscles exhibit electrically excitable membrane responses and are innervated by motor neurones lacking spontaneous activity. The tonic muscles, on the other hand, show only graded junctional potentials and are innervated by motor neurones that show spontaneous activity.

The crayfish uropods, which are the paired terminal appendages, have about 20 muscles whose contraction brings about complex movements of the uropod [3]. The anatomy of the uropod musculature was first described in the *Astacus* [4]. Larimer and Kennedy [1] revised it for *Procambarus,* and described the phasic/tonic classification of the uropod muscles together with their innervation from the abdominal ganglion. They described a functional separation of the motor neurones especially in the second and the third root from the sixth abdominal ganglion. However, their work on both the classification and the innervation of the musculature is incomplete and misleading.

Although Takahashi and Hisada [5] recently reported the fiber types of uropod muscles by the myofibrillar ATP-ase histochemistry and also the innervation of slow muscles by electrophysiology, incompleteness has still remained especially in the innervation pattern.

This paper describes the innervation of the tonic uropod muscles, whose motor neurones travel in either the second or the third root of the sixth abdominal ganglion, based on an electrophysiological study.

MATERIALS AND METHODS

Experiments were carried out at room temperature (about 20°C) on the isolated abdomens of adult crayfish, *Procambarus clarkii,* which were pinned with their ventral side up in a chamber filled with physiological saline [6]. The ventral cuticle of the sixth abdominal segment and the protopodite were partially removed to expose the sixth abdominal ganglion. The ganglionic roots and the muscles were then examined. Ganglionic roots other than those of the second and the third roots were cut off close to the ganglion. An

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Table 1. Tonic muscles of crayfish uropod and their innervation

Tonic muscles	2nd root	3rd root
Flexors		
Telson-uropodalis anterior	2E	
Telson-uropodalis lateralis		2E, 1I
Slow bundle in Telson-uropodalis posterior	3E	1I
Rotators		
Medial rotator	1E	1I
Promotor		
Abductor exopodite ventral		2E, 1I
Remotors		
Reductor expodite	1E	1E, 1I
Adductor exopodite accessory muscle	1E	1E, 1I
Adductor endopodite dorsal		1E, 1I
Adductor endopodite ventral	1E	1I

Excitatory (E) and inhibitory (I) motor neurones through the second and the third root are each shown with their probable number.

oil-hook electrode was used for extracellular recording of motor root activity or stimulation. Intracellular recordings from muscle fibers were made with 2 M K-acetate filled glass microelectrodes (10–20 Mohm in resistance).

RESULTS AND DISCUSSION

Nine muscles shown in Table 1 were recognized as tonic muscles which were innervated by motor neurones in either the second or the third roots of the sixth abdominal ganglion. The shape and position of these muscles are referred to the works of Schmidt [4], Larimer and Kennedy [1], Newland [7], and Takahashi and Hisada [5]. Table 1 also describes the innervation of the muscles. A continuous barrage of spontaneously occurring excitatory junctional potentials (EJPs) were consistently recorded in all of these muscles. Light mechanical sensory stimulation of the uropod caused an augmentation of EJPs, never resulting in active responses (data not shown). Spontaneous inhibitory junctional potentials (IJPs) were occasionally observed in a few muscles.

Simultaneous recordings of continuous EJPs or IJPs in the muscle fiber and motor root activity close to the ganglion offered direct evidence of the innervating root when individual EJPs or IJPs could be associated with the activity of an identifiable motor neurone (Fig. 1). Multi-unit motor activity of a root, however, often made it difficult to pick up single unit activity corresponding with the individual EJPs even if the unit seemed to be in a given root.

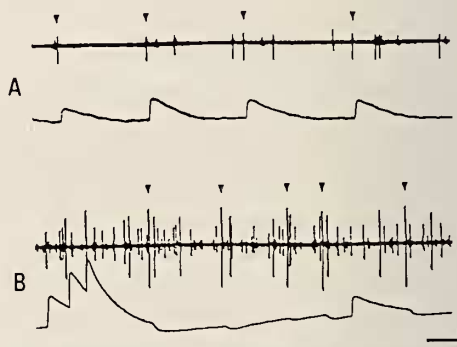

Fig. 1. Simultaneous recordings of the motor root activity and muscle responses. (A) EJPs of the adductor endopodite ventral and identifiable motor neurone activity (arrowheads) in the second root. (B) IJPs of the adductor endopodite dorsal and identifiable motor neurone activity (arrowheads) in the third root. Calibration: 100 ms, 5 mV.

Cutting either the second or the third root offered indirect evidence of the innervating root especially with the excitatory innervation that was spontaneously active and consistent. The remaining EJPs in a muscle fiber during the course of such an experiment indicated that the motor neurone innervating the muscle fiber was not included in the cut motor root. The motor neurone was in the motor root remaining intact. One of the examples is shown in Figure 2. Spontaneous EJPs in the muscle fiber of the reductor exopodite suggested that there were probably two excitatory motor neurones innervating this muscle. Cutting the second root resulted in a single type of EJPs. Using a different preparation, cutting the third root, while the second root remained, also produced a similar result. These results show that one of the two excitatory motor neurones innervating the reductor exopodite is in the second root, and the other is in the third root.

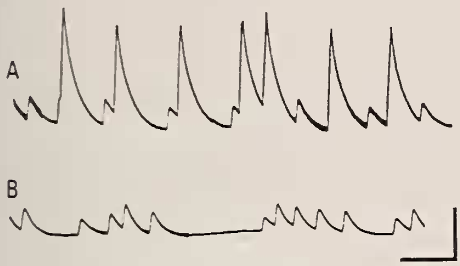

Fig. 2. Double excitatory innervation of the reductor exopodite through the different roots. (A) Large and small spontaneous EJPs. (B) Small EJPs still continue after cutting the second root. Calibration: 100 ms, 5 mV.

Separate electrical stimulation of the proximal cut end of the second or the third root was a useful means to determine the innervation pattern, especially with regard to the innervation of inhibitory motor neurones which usually showed no spontaneous activity. In the muscle fibers of the adductor endopodite dorsal, for example, a train of five stimuli (50 μs rectangular current pulse with 5 ms interval) to the second root evoked no response, while that to the third root evoked both EJPs and IJPs depending on the stimulus intensity (Fig. 3A). This demonstrates that both the excitatory and inhibitory motor neurones innervating the adductor endopodite dorsal are in the third root. In another example of the adductor endopodite ventral, a similar stimulation to the second root evoked the summating EJPs, while that to the third root evoked the summating IJPs (Fig. 3B).

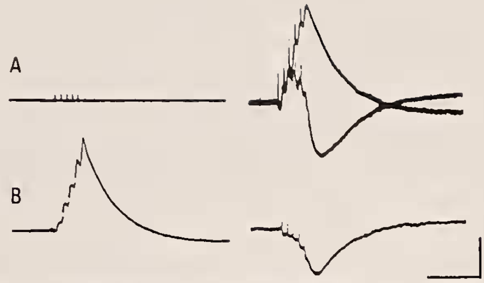

Fig. 3. Separate electrical stimulation of the root and the muscle responses. Responses of the adductor endopodite dorsal (A) and the ventral (B) to the second root stimulation (left column) and the third root stimulation (right column) respectively. In the right column of A, summating EJPs and IJPs at higher and lower stimulus intensity respectively are superimposed. Calibration: 50 ms, 2 mV.

This demonstrates that the excitatory and inhibitory motor neurones to this muscle are in the second and the third root, respectively.

Similar patterns of innervation of the uropod tonic muscles were obtained by each of the three types of methods described above. However, slight ambiguity with regard to the number of excitatory motor neurones innervating a muscle through the same root still remains. A possibility of the presence of the inhibitory innervation of telson-urpodalis anterior also still remains.

Although Larimer and Kennedy [1] described the telson-uropodalis anterior, lateralis and posterior as being phasic muscles, the former two were regarded as tonic muscles, and the latter was regarded as mixed muscles in this study. Similar results were reported by Takahashi and Hisada [5]. The anal dilator, which is also a tonic muscle, is not listed in Table 1, since its motor neurones (two excitatory and one inhibitory neurones) are included in the sixth root.

The work of Larimer and Kennedy [1] described two main features of the innervation pattern of the tonic muscles. Firstly, that all motor neurones innervating a certain muscle are in the same ganglionic root. Secondly, that there is a clear functional separation of the second and the third roots of the sixth abdominal ganglion. The motor neurones in the former innervate the flexors, telson-uropodalis group, and all of the remotors. Those in the latter, on the other hand, innervate the promotors. In this study, however, these characteristics were not observed. Excitatory motor neurones innervating a certain muscle were not necessarily included in the same root, as shown in the reductor exopodite and adductor exopodite accessory muscle. Moreover, in several muscles, the excitatory and inhibitory motor neurones were included in different roots. Newland [7] has reported a similar result on the innervation of the medial rotator.

The most characteristic feature of the innervation pattern of the uropod tonic muscles through the second and the third roots of the sixth abdominal ganglion was that all of the inhibitory motor neurones were in the third root. The second root contained purely excitatory motor neurones. The third root, on the other hand, contained both the excitatory and inhibitory motor neurones. Several motor neurones do cross from one root to the other on the way to the target muscle [7]. It is not clear in the work of Larimer and Kennedy [1] where the recordings of motor root activity or stimulations were performed. The recordings or stimulations near the target muscle, not close to the ganglion, may lead to inaccuracies in the determination of the innervation pattern. In the present, there is no evidence of common innervation of the excitatory or inhibitory motor neurone between any two or more muscles that are anatomically separate [8]. It may be necessary to examine the detailed course of the individual motor neurone from the ganglion to a target muscle by means of a histological investigation as well as further electrophysiological investigation.

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